

FIG. 1

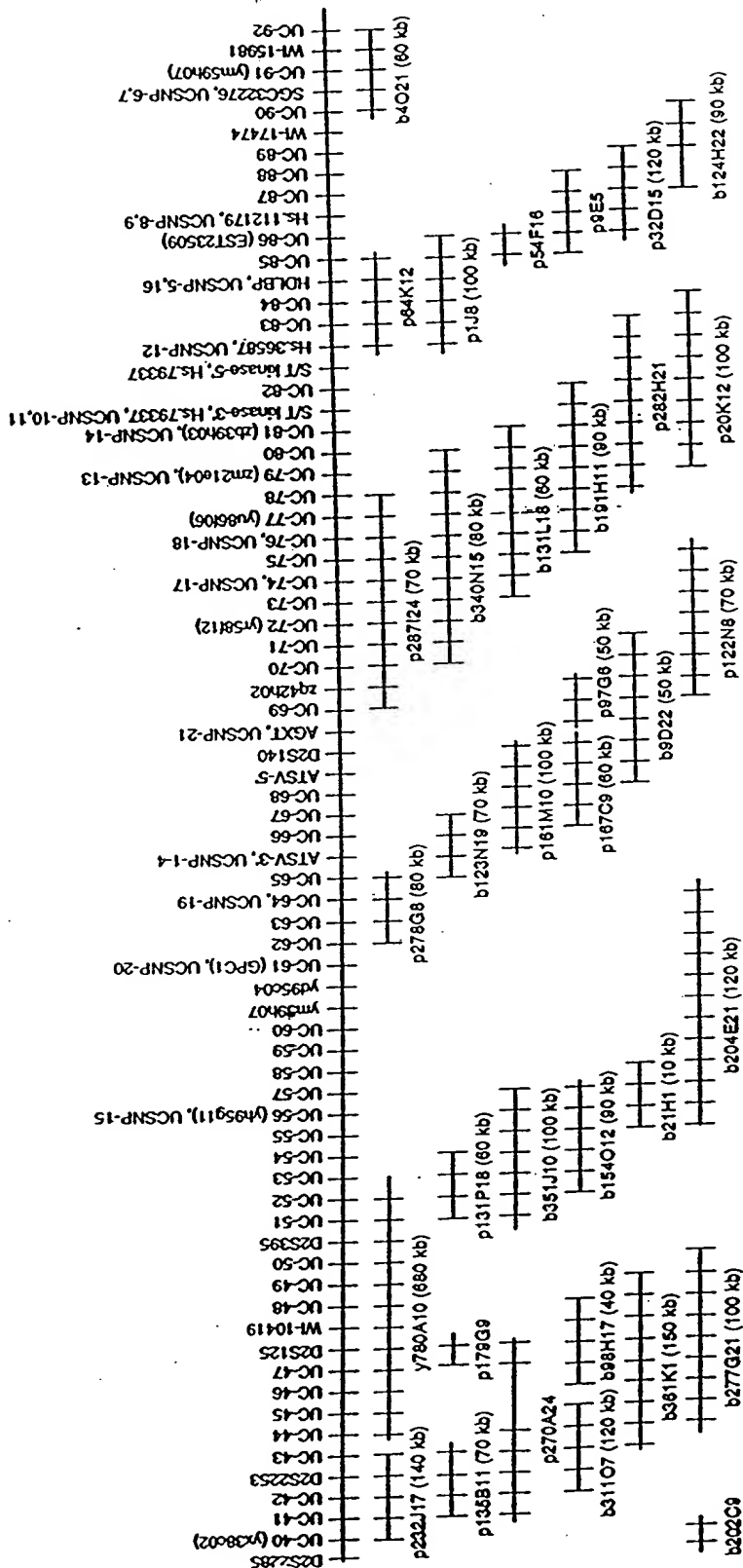


FIG. 2

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FIG. 3

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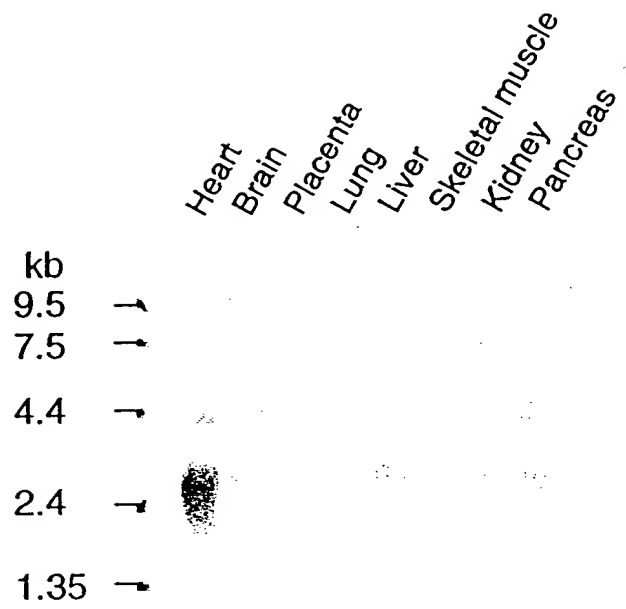


FIG. 4

Domain I ← → Domain II

bcAPN5 -----HFCVCKPTEDQNTYALRQOCRRKRVLFEDPLFPAIDDSLYY-KCTGCP-----AVYMKRPKGCIGEDPRFLVDG 67
bcAPN6 -----HFPLALFRNKQYQELKQOCNDRGLFCDFPLPNDLSFFNRLLPC-----KVYMKRQDISDOEHLVGN 67
bcAPN7 NPTVISASVAPRTAAEPRSPGVYHHPAQSKATEAGGQNPSCGYSAIISRNPTIIGVKKETFDQKRLCKLVYVDPEFPDGTSLYTSQKFP-----IQYMKRPPEICENPFRIIDG 115
bcAPN9 NPTLYT-----RAPGCPMPVP-----EDARHTSSQGFENRQOCQGLCTGFEDAFANSSFLATSRFPQ-----IPYMKRPEICVKNPFILOG 63
bcAPN1 -----HSEELITTVYTCGVSAQVQKQAREGLG-----RHEALIKVLQOQDQVLRVCLQSGTFRDEAFAPVPQSLGYKDLGNSSKTYTGDKRPFTELLSNQPIVDG 101
bcAPN2 -----HAGTAAKLAIDREAAEGLG-----SHERAIKVLQOYALRNDCELGTLFQDPSFPATPAGFELGELCTPTSSKTRGHRKRPTEICADPQFIIG 91
bcAPN8 -----HAAALAGVSKQRAVADGLG-----SHQNAVKTLOQDETARQCLASGVLEKDPQEPACPSALGYKDLGPGSPDTGCVIMKRPTELCNPFQIVG 59
bcAPN10 -----HRAAGCATPARELFRDAAFPAADSSFLCDSLSTVLAVERATIMRQPEIQDCAITRPLFDG 21

bcAPN5 ISSKDLIQQGVQNCVFVAACSSSLAKRESLAKQVIFPKMEDQMDPRKACAYAGIFHFHFVRLG-HVDVYDLERLPTVNNQLITCHSRSRNEFWALVERATYAKLAGCTQALDQCHTADAL 186
bcAPN6 ISSNQLIQIGRLGNKAHISAFSLAVQESHRNKAIPNEDQMDPRPEKTYAGIFHFHFVRLG-HVDVYDLERLPTVNNQLITCHSRSRNEFWALVERATYAKLAGCTQALDQCHTADAL 186
bcAPN7 ANRDTIQCGELGDCWFLAAJLACTLTHQLLVFIP-----BDGSPCTEYAGIFHFHFVRLG-HVDVYDLERLPTVNNQLITCHSRSRNEFWALVERATYAKLAGCTQALDQCHTADAL 186
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bcAPN2 ATRTDIQCGELGDCWFLAAJLACTLTHQLLVFIP-----BDGSPCTEYAGIFHFHFVRLG-HVDVYDLERLPTVNNQLITCHSRSRNEFWALVERATYAKLAGCTQALDQCHTADAL 186
bcAPN8 ATRTDIQCGELGDCWFLAAJLACTLTHQLLVFIP-----BDGSPCTEYAGIFHFHFVRLG-HVDVYDLERLPTVNNQLITCHSRSRNEFWALVERATYAKLAGCTQALDQCHTADAL 186
bcAPN10 PREGQVQGLGDCWFLCACAALQKSRLLDQVTP-----PQPSWADQETGNSFTCRWQPGKRVVETTDRLCLAGRLCFSRQOQREDWFLPLLEKTVAKVGSYEHUAGQVADALV 175

bcAPN5 DFTGCVSEPIDLTGCGFANDETQRKRLFERMLKLVHSRGLSISAKV-----TAAKH-----EARLAGLTKGHYATVDV 258
bcAPN6 DFTGCVLAIEDHQGRNTDLVEEKHLPCGLVYRTKGLGLCSIESP-----SQEDQ-----EVETDQKGLGTYHTTID 259
bcAPN7 DFTGCVAAEFELRD-----APSDNKKDKKATERSGLQCSIDGQNTNITGTSPSGLAKRGLVGLNVRNHSLSLQCSLDPPGSRDEPRTITIPVQVTEHRAOGLVRGHASVTVGL 341
bcAPN9 DFTGCVAAEFELRD-----APSDNKKDKKATERSGLQCSIDGQNTNITGTSPSGLAKRGLVGLNVRNHSLSLQCSLDPPGSRDEPRTITIPVQVTEHRAOGLVRGHASVTVGL 341
bcAPN1 DFTGCVAAEFELRD-----APSDNKKDKKATERSGLQCSIDGQNTNITGTSPSGLAKRGLVGLNVRNHSLSLQCSLDPPGSRDEPRTITIPVQVTEHRAOGLVRGHASVTVGL 341
bcAPN2 DFTGCVAAEFELRD-----APSDNKKDKKATERSGLQCSIDGQNTNITGTSPSGLAKRGLVGLNVRNHSLSLQCSLDPPGSRDEPRTITIPVQVTEHRAOGLVRGHASVTVGL 341
bcAPN8 DFTGCVAAEFELRD-----APSDNKKDKKATERSGLQCSIDGQNTNITGTSPSGLAKRGLVGLNVRNHSLSLQCSLDPPGSRDEPRTITIPVQVTEHRAOGLVRGHASVTVGL 341
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Domain II ← → Domain III

bcAPN5 RKVRLGHLGLAFAFFSKELDIRLRNHPGCRKMGKQWSDTSEEMQVKSSEKREKHGVTVDGGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN6 RKRLRGLRGLVNTSTKLYVRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN7 DEVFP-----RGEKVLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN9 KQVFP-----RGEKVLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN1 KQVFP-----RGEKVLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN2 EEVFN-----HGSGLQLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN8 EEVFN-----HGSGLQLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373
bcAPN10 RELQG-----QAGQCLLLRLNPLRGLKQVSGWSEISEEMQVLTDRKLNGLVSGDGEFMTEDVCRVYTDIICRVNTHSL-SHKTMEEARL-----HGAMLTDEHPQNRG 373

bcAPN5 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN6 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN7 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN9 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN1 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN2 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN8 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472
bcAPN10 QCCINRBDITFFNQPIYFVEMVKK-----EVLCIQQRPRSTRDRGKGNLAIGFDLYKE-----E-HRQYRHSLSL-QHKAASSITINSRVLRTADQDQRYVITPT 472

Domain III ← → Domain IV or T

bcAPN5 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN6 MFQGRSTSEFLRLVFEAPVQVLELTDPMHS-----C-----HSLARGYKPVVTVTVISAGDEKKRY-----532
bcAPN7 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN9 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN1 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN2 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN8 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536
bcAPN10 TFEPTGCTGFLRVFTDVRSNCRALDEPPT-----C-----HSSLOCTQLVTVQVYVGLAGLKD-----536

bcAPN5 -----SPTGANSVYIKCQGVKVSVAQK-----TSTPEVNVKGFIRKLSQPIITVQVGR-----VLKDEFLQVHLKADPQVQALHTLHLDNRISQ 618
bcAPN6 -----MNETVYPIILIKCQGVKVSVAQK-----TVHAISTDQAIYFRTDITPIIIVQVGR-----KFCDDQVQVHLKADPQVQALHTLHLDNRISQ 620
bcAPN7 VVNGKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679
bcAPN9 VLQKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679
bcAPN1 VLQKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679
bcAPN2 VLQKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679
bcAPN8 VLQKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679
bcAPN10 VLQKIDIRKGLFSLSCSIRVNLDRDQSGKLNIFERHLAKLAKAMQIFKQYTDQSGTINSTRHNRVADGCVLQVYDITIRYADNRIDUSFTQCVLEGRHAFH 679

bcAPN5 PSNLPGTVAVHILSSLSMAV-----639
bcAPN6 ARVKQGHISPKVSISSDQTEL-----641
bcAPN7 DRQDQGIIRKLVNLEQLQTHA-----821
bcAPN9 STNKEFIRHNLINERIKLTHN-----690
bcAPN1 DTDLQGVVTFDLKVLQTHA-----714
bcAPN2 DPENTGTIDELVSLWCLFSL-----700
bcAPN8 DRQDQGIIRKLVNLEQLQTHA-----703
bcAPN10 EHLQGFQGVSVNAYKT-----672

FIG. 5

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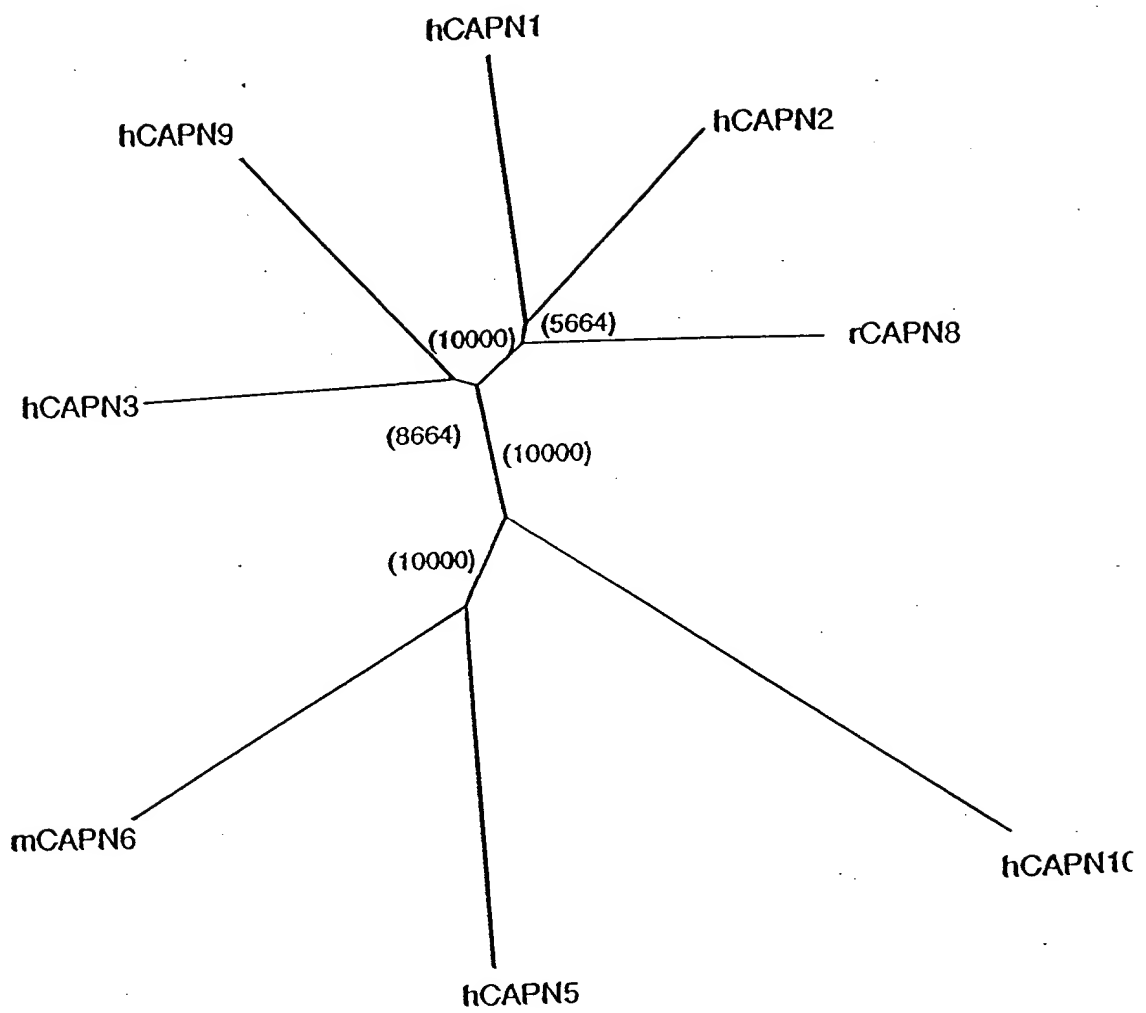


FIG. 6

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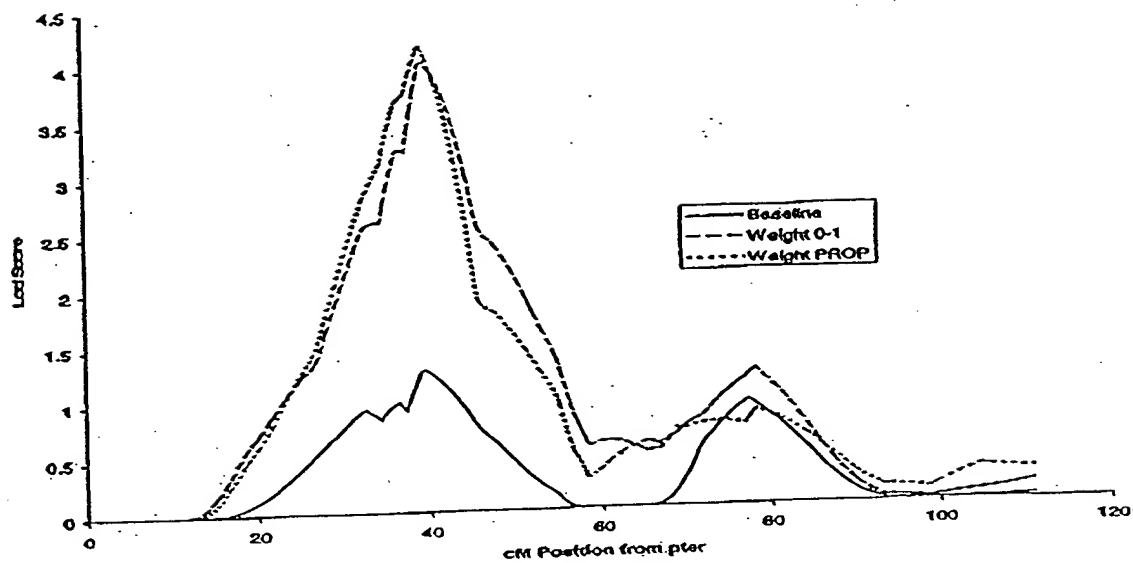


FIG. 7A

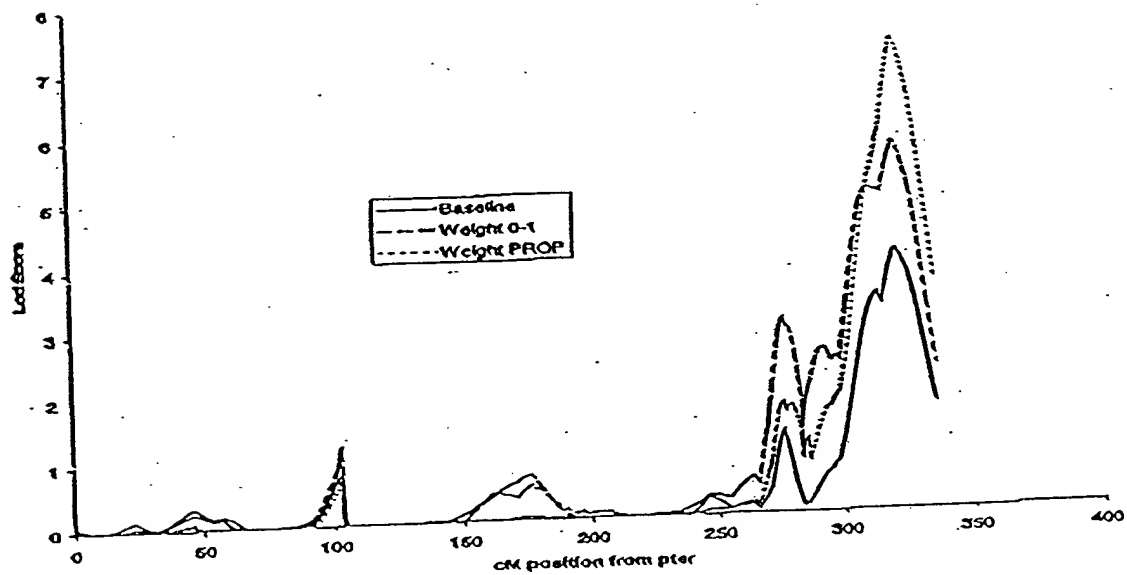


FIG. 7B

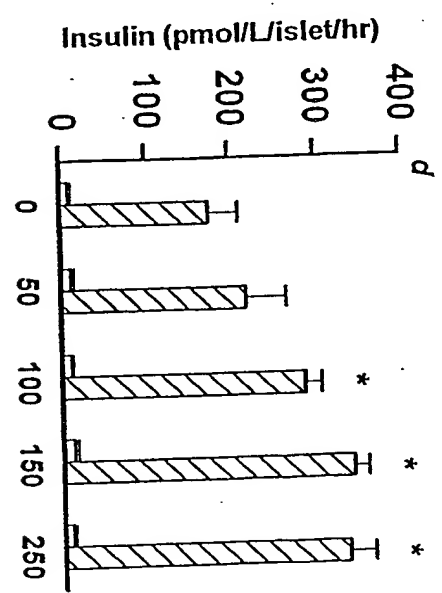
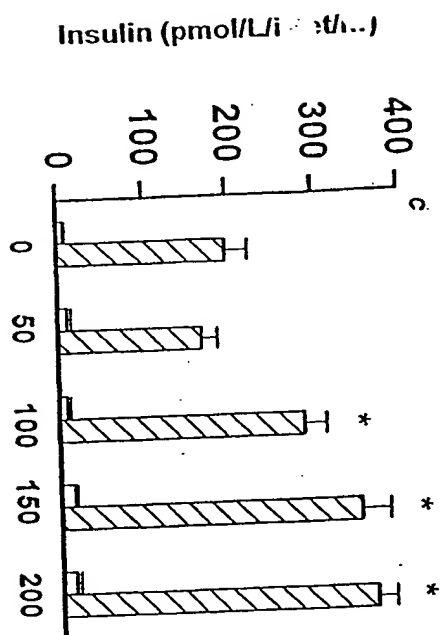
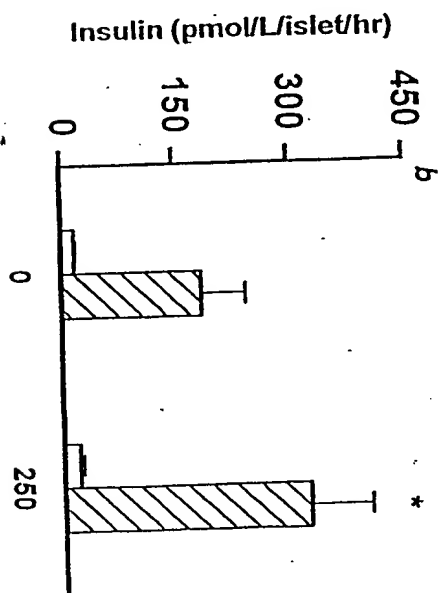
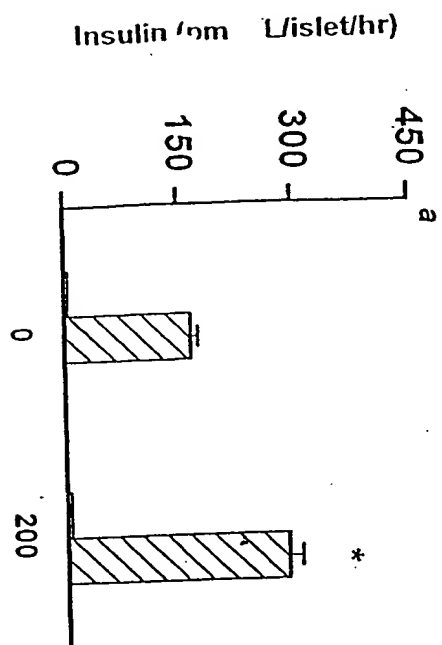


FIG. 8

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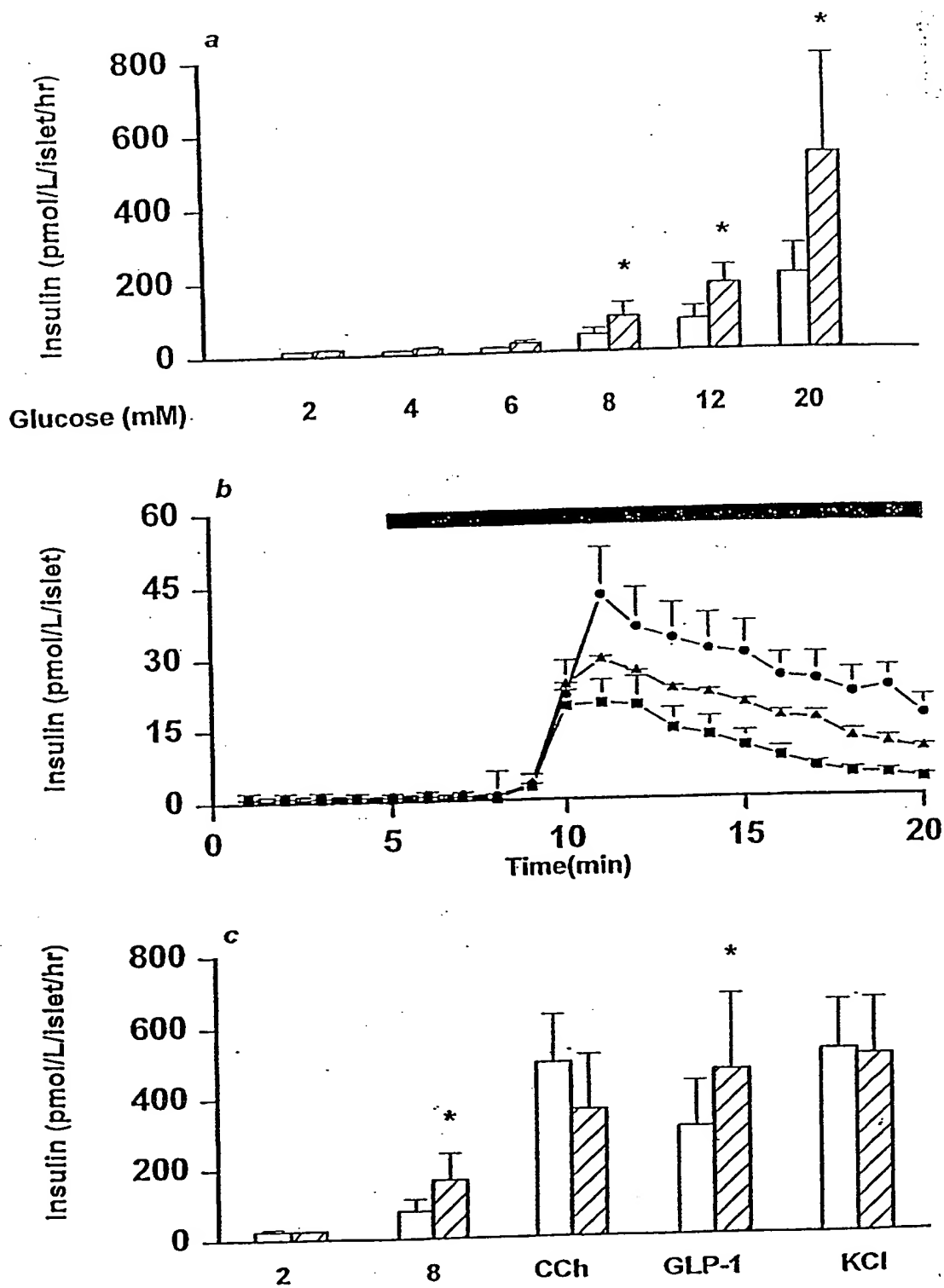


FIG. 9

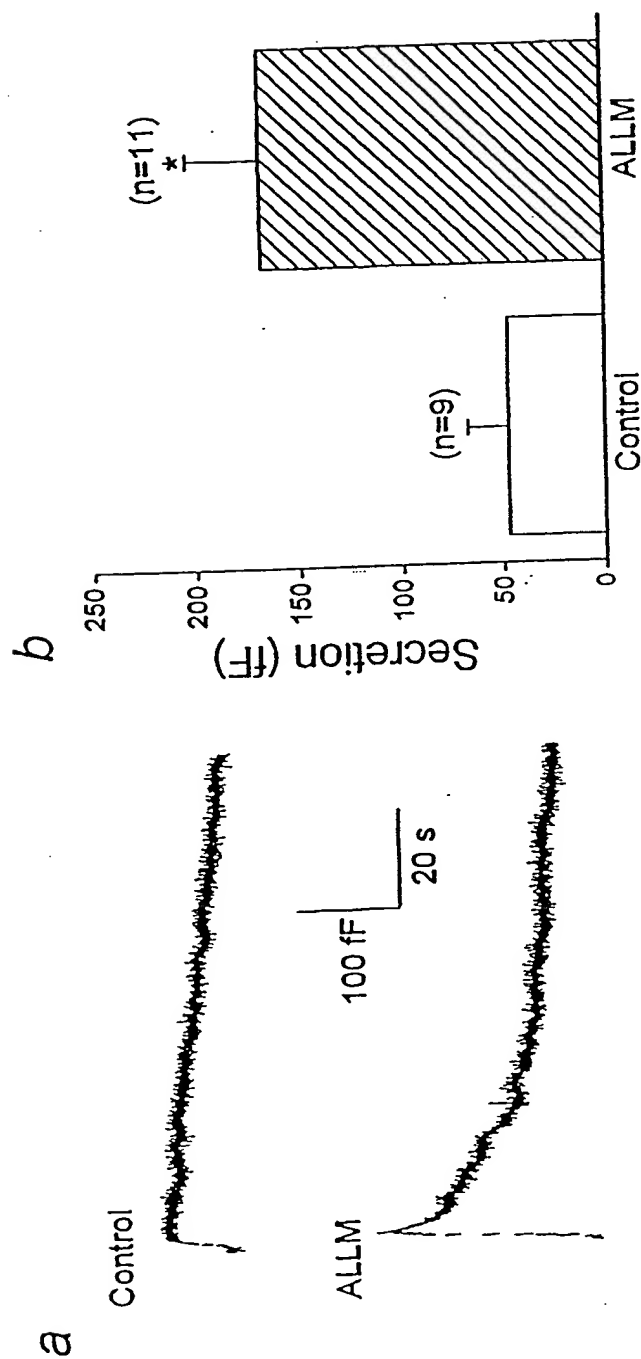


FIG. 10

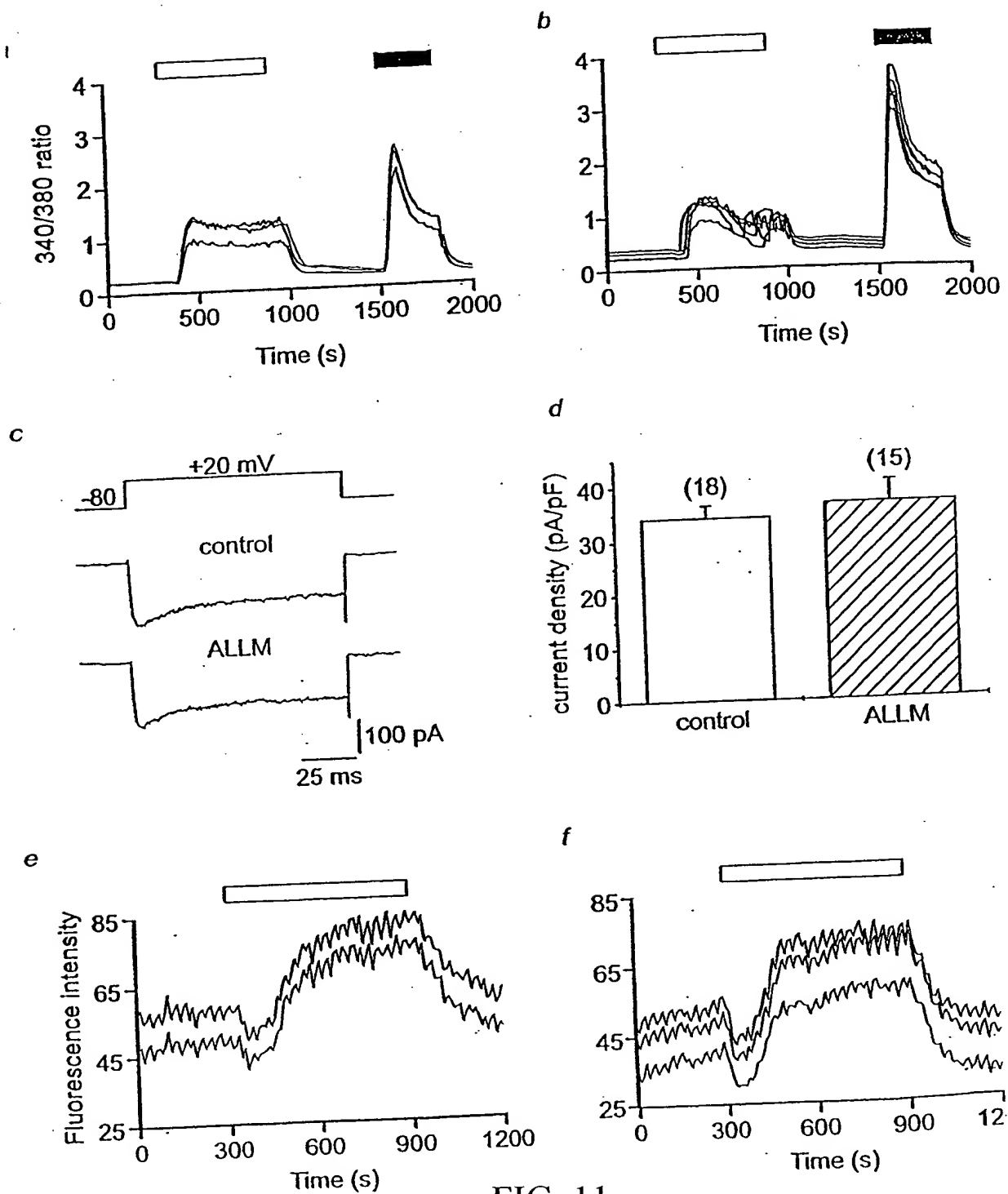


FIG. 11

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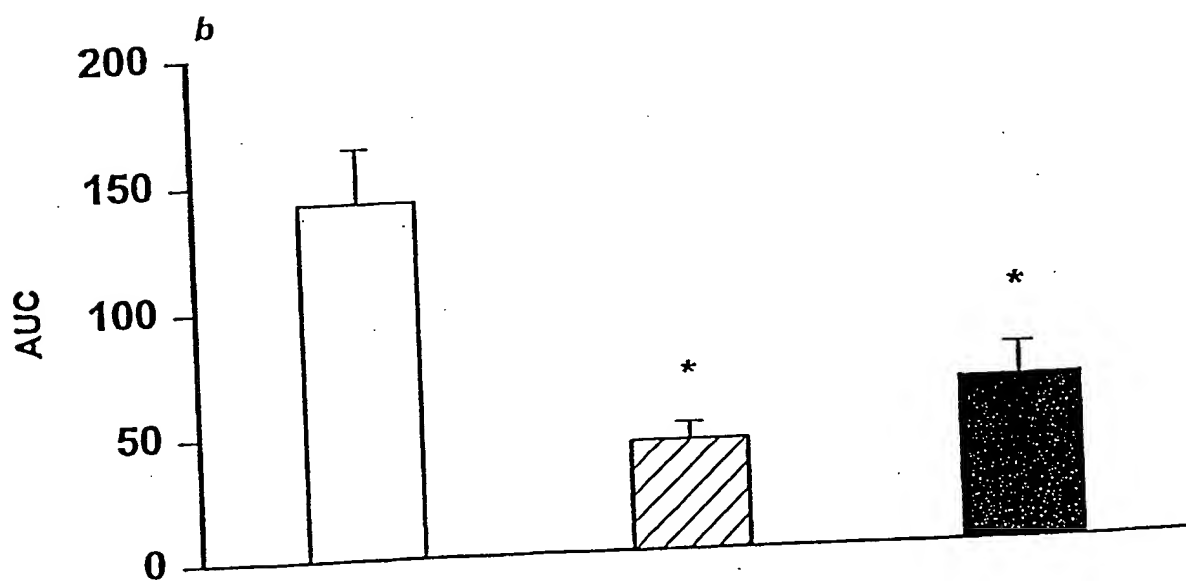
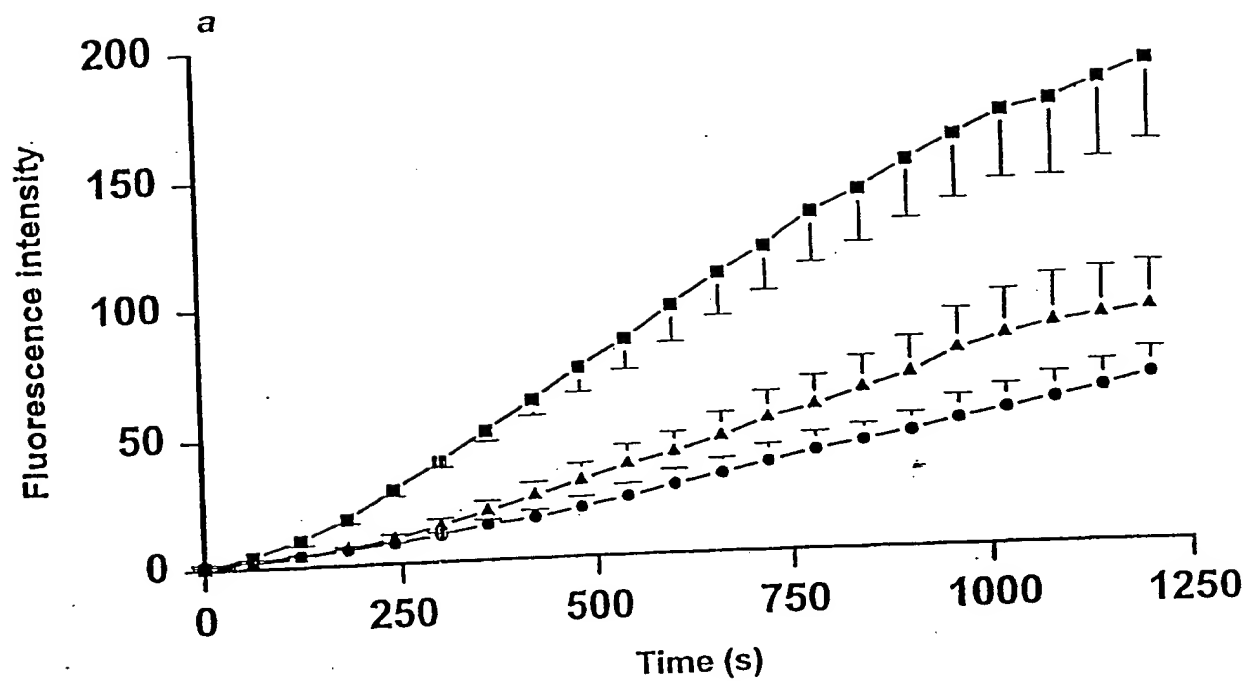


FIG. 12

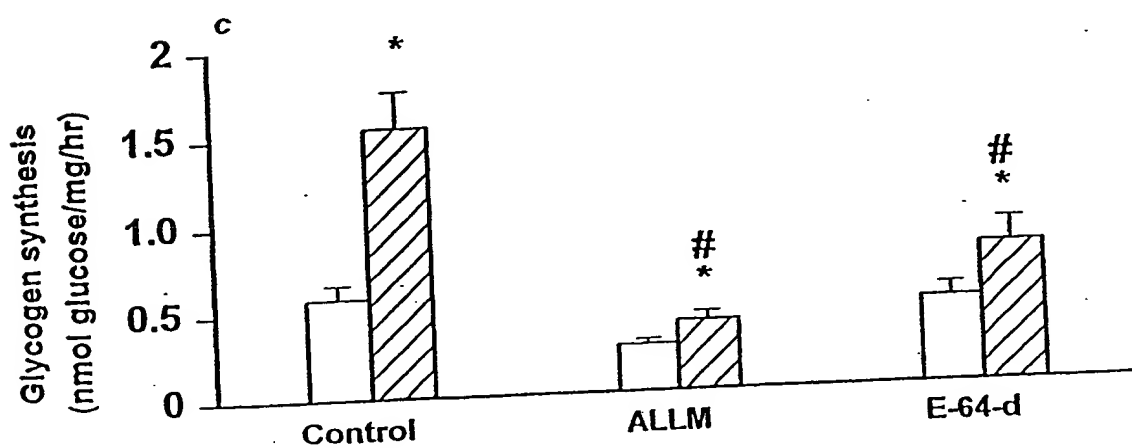
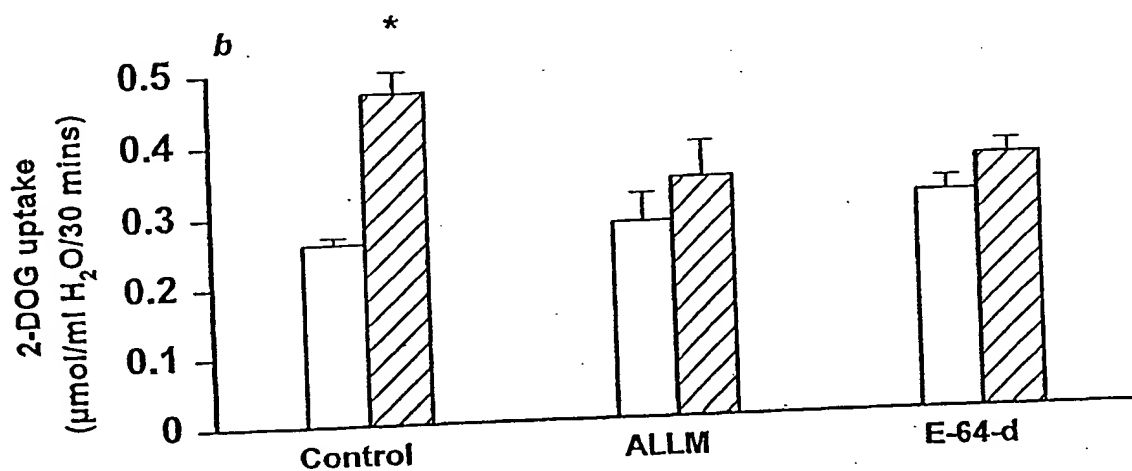
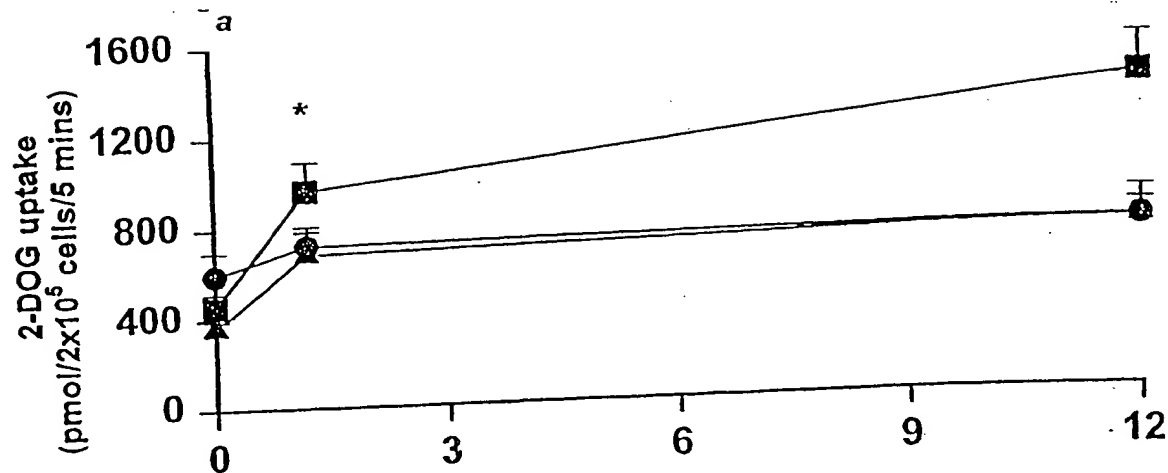


FIG. 13

Fig14. Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion

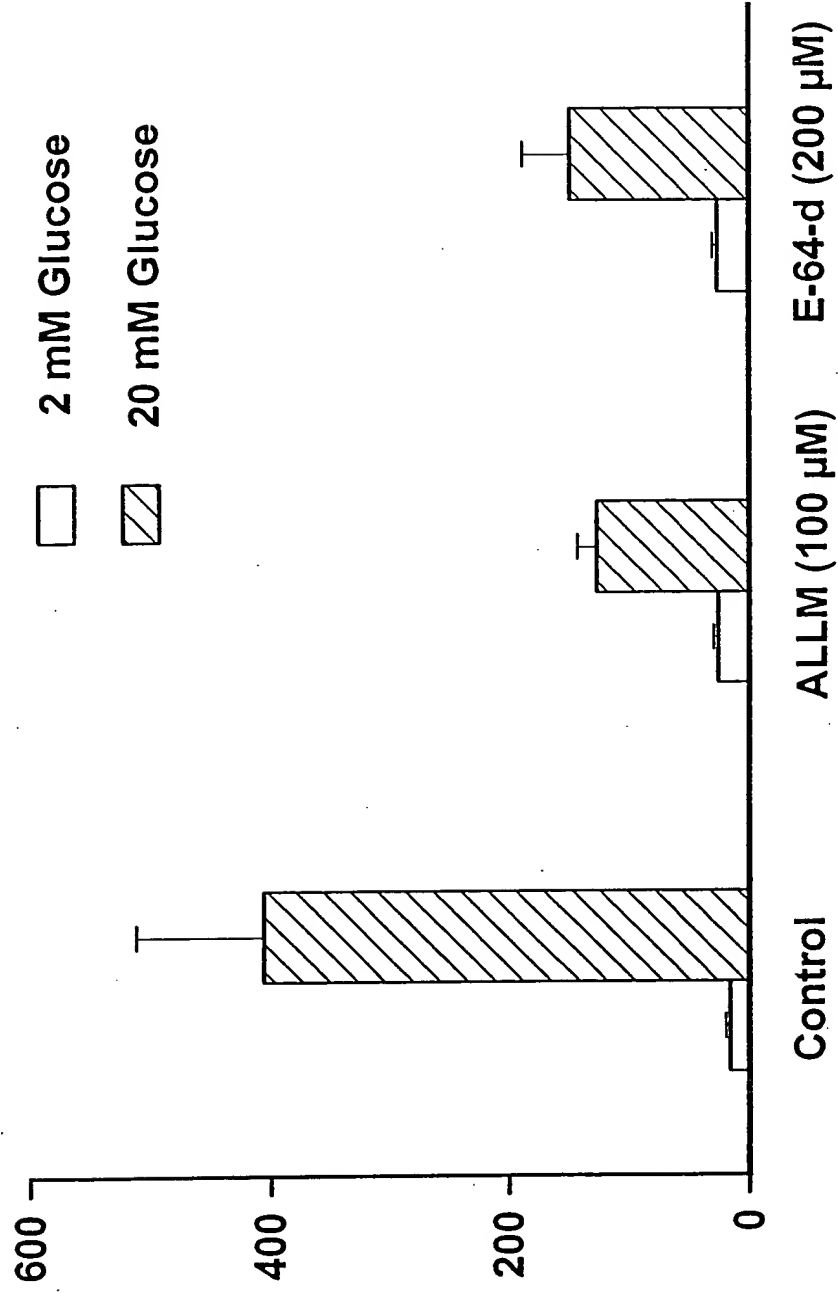


Fig 15 Insulin content in 48 hour cultured islets (n=4)

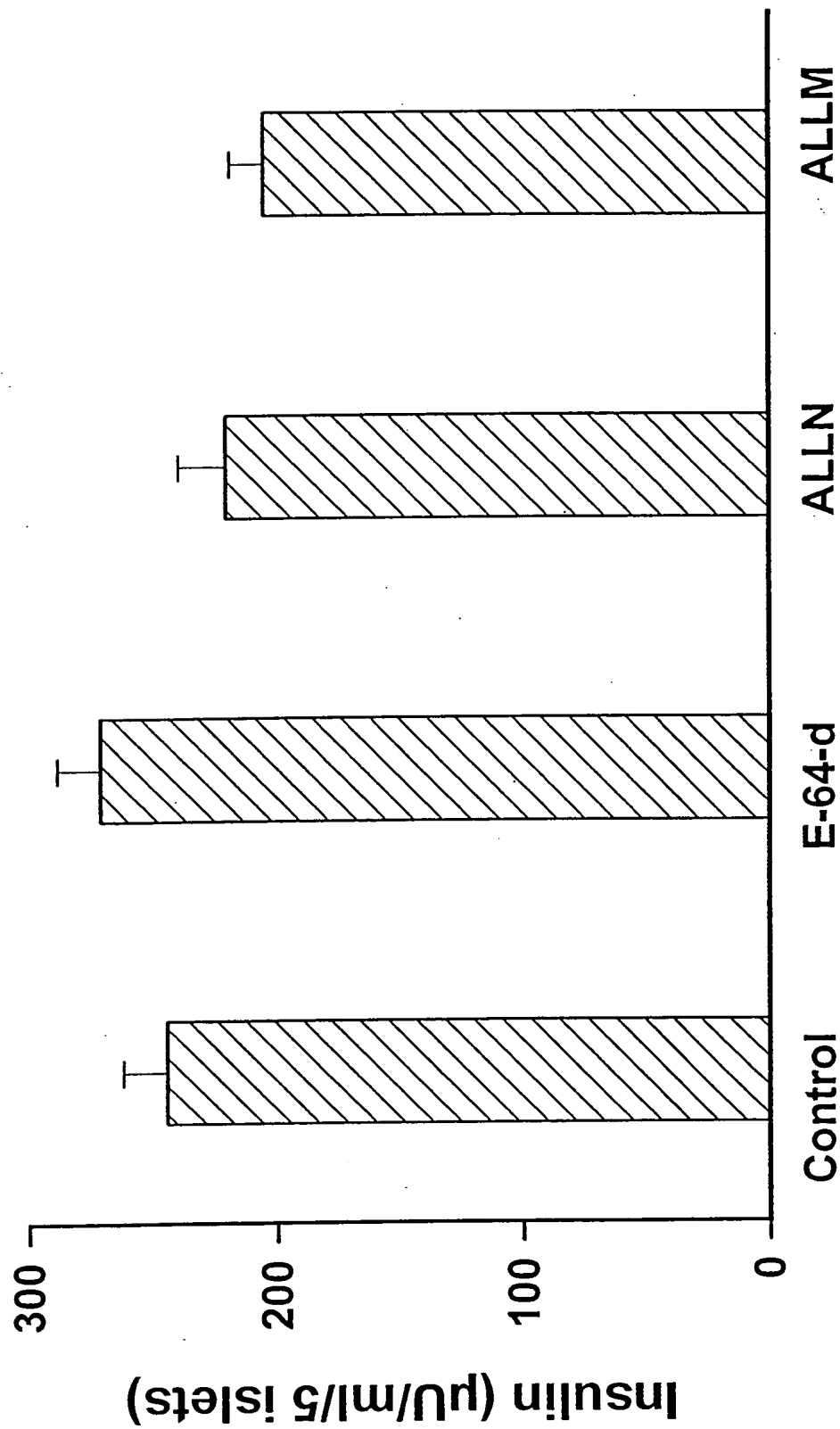


Fig16. ALLM dose response in 48 hour treated islets

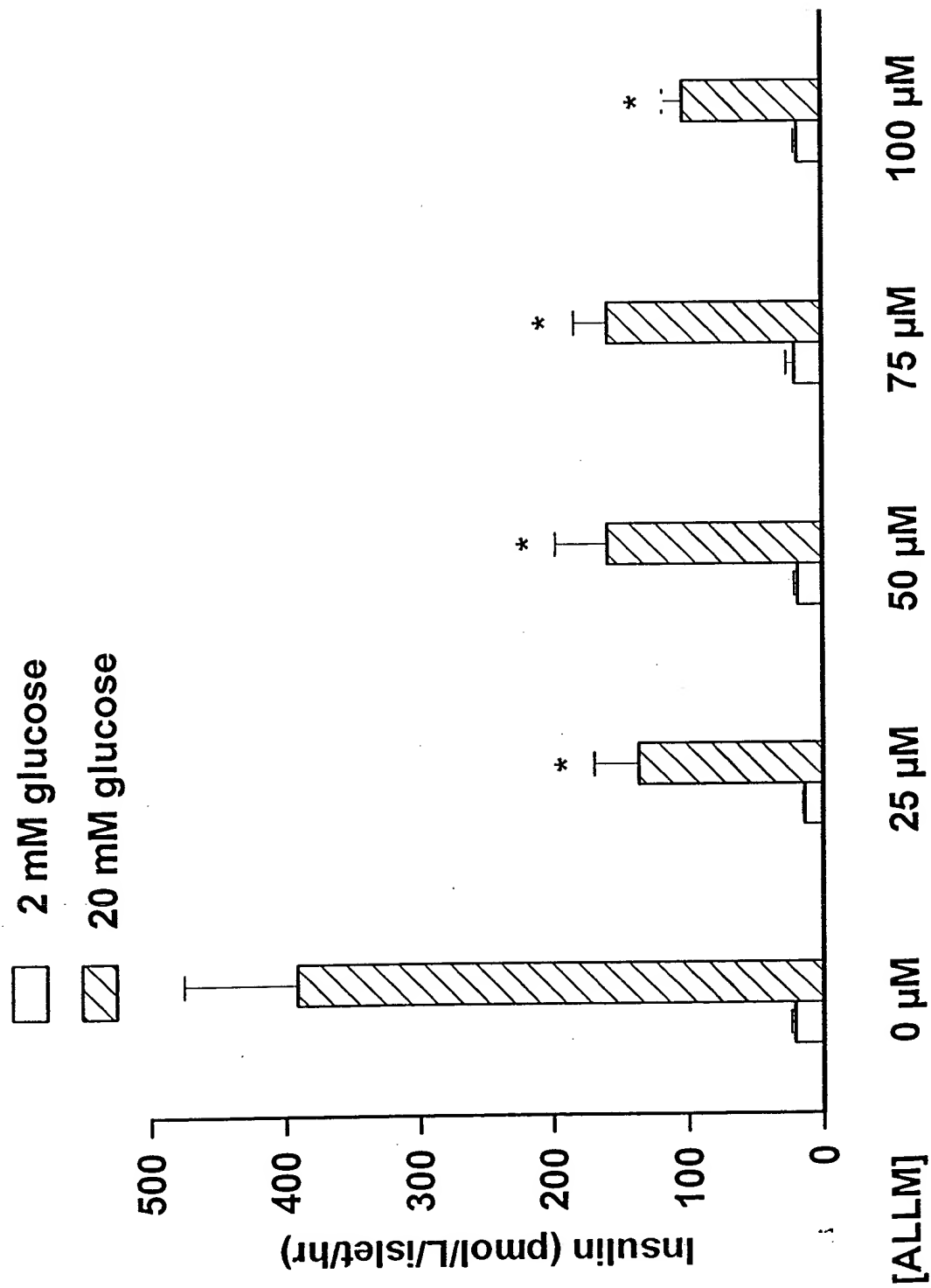
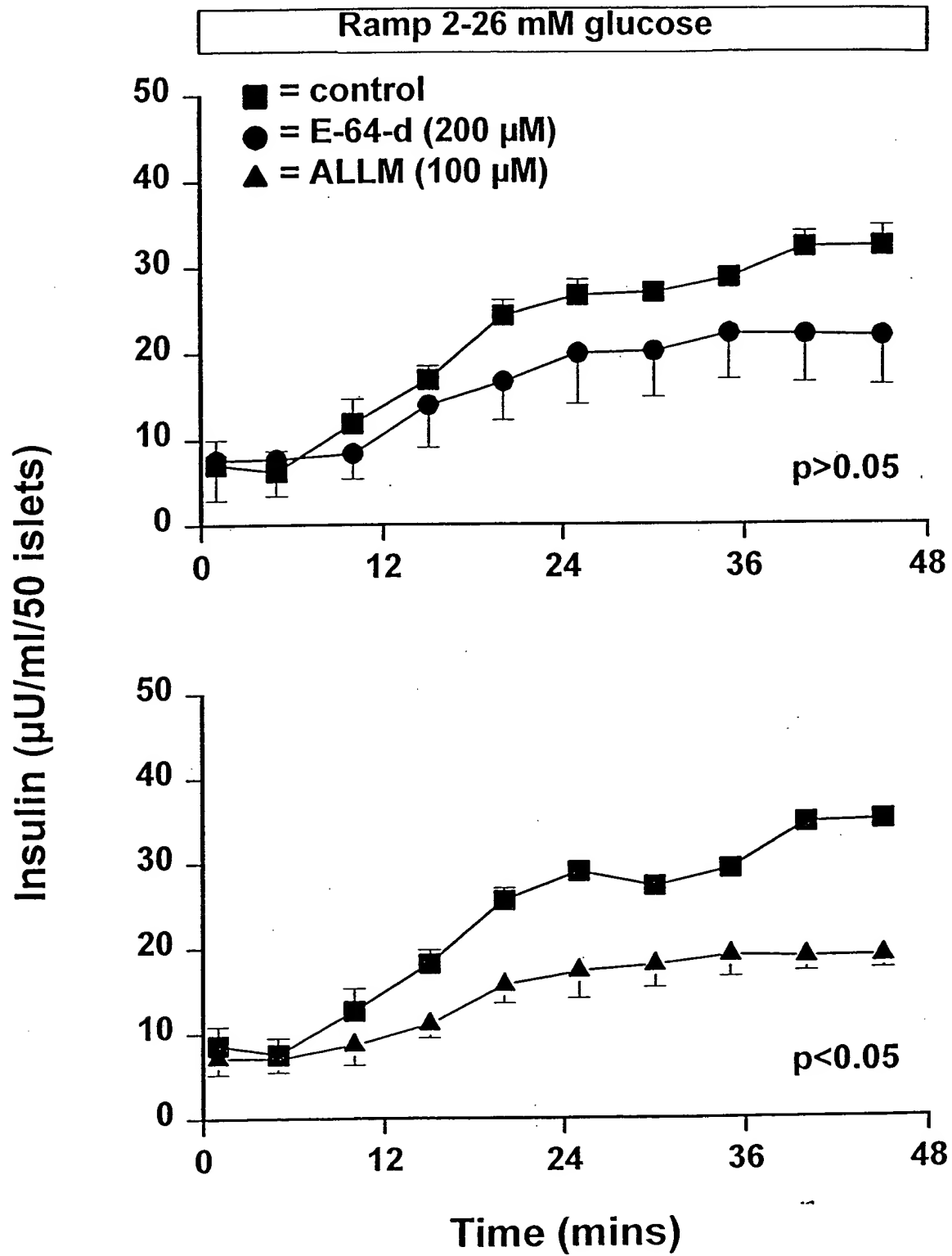


Fig17. Perifusion of 48 hour cultured islets (n=4)



Insulin secretion in ALLM or E64-d treated mouse islets: Reversal study

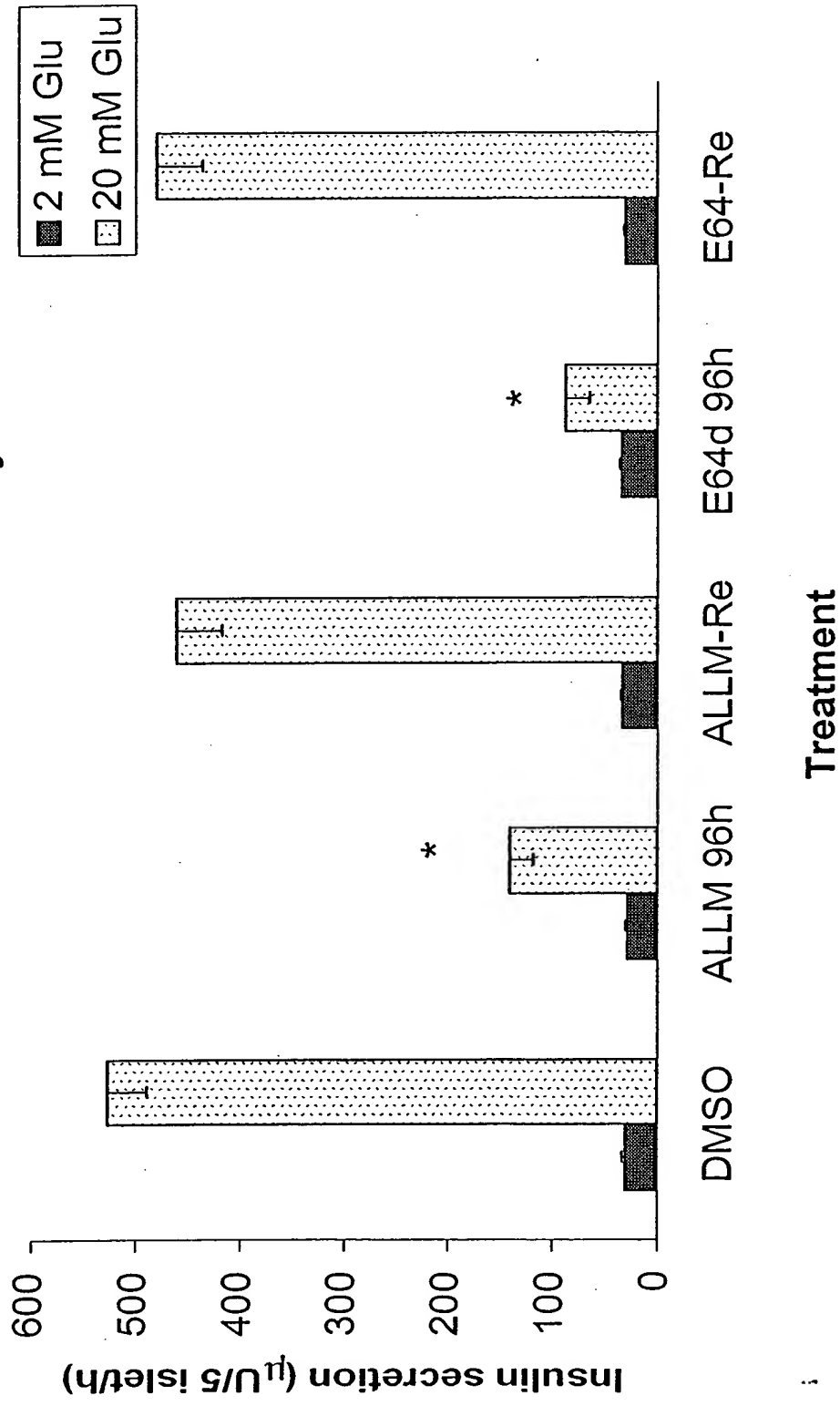
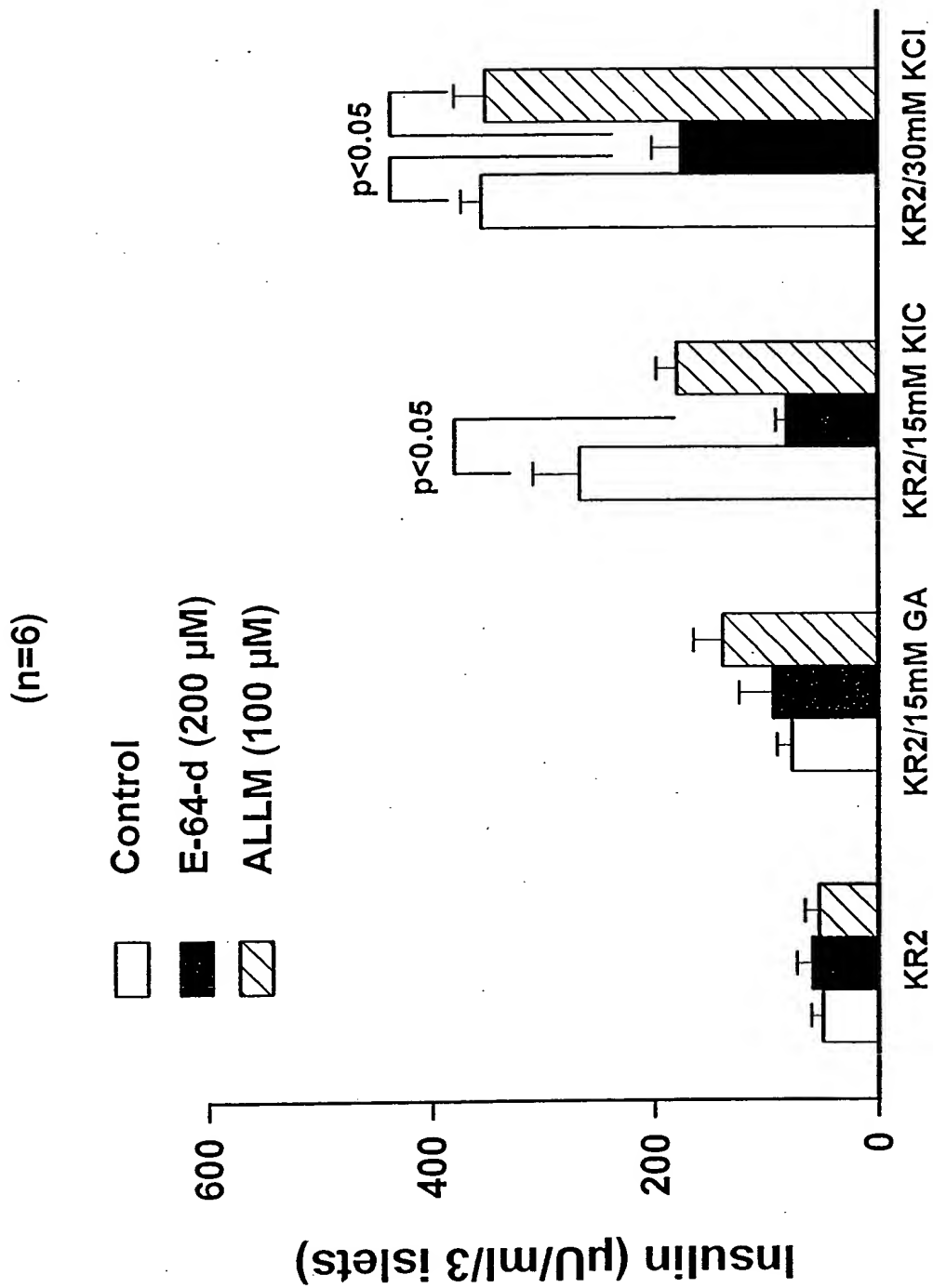


Fig. 18

Fig19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs



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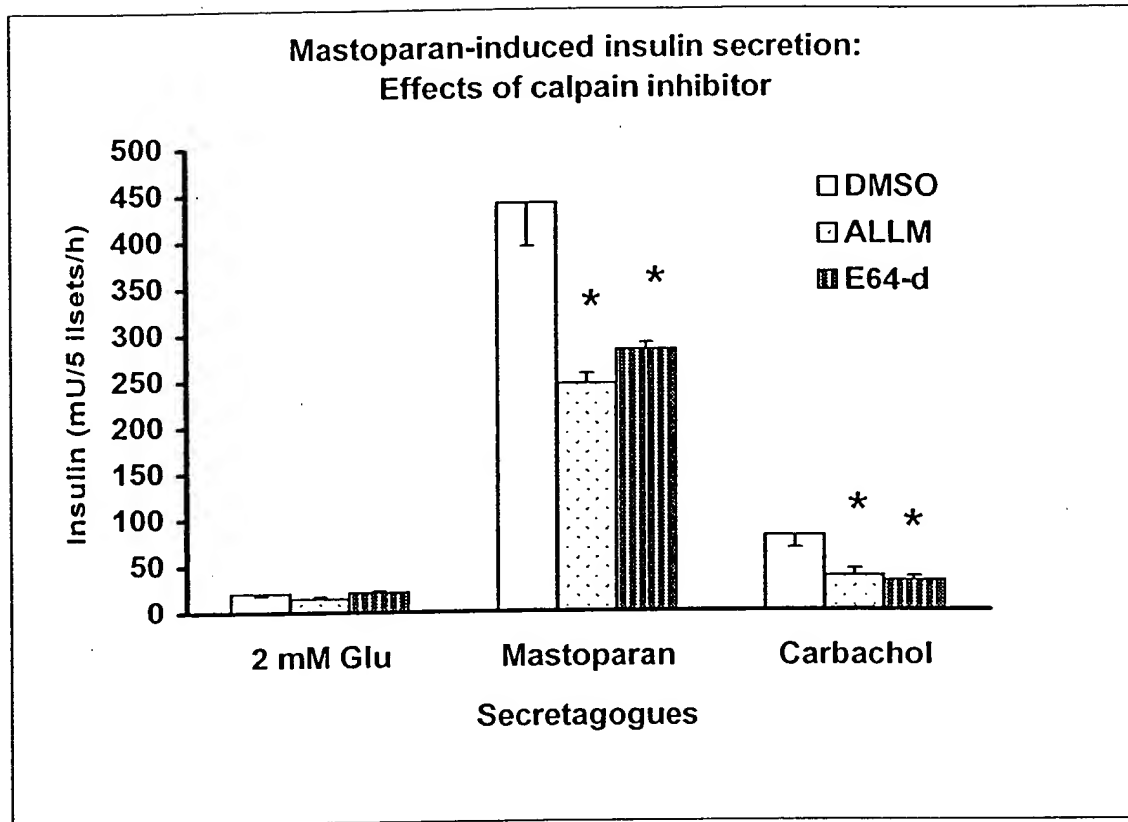
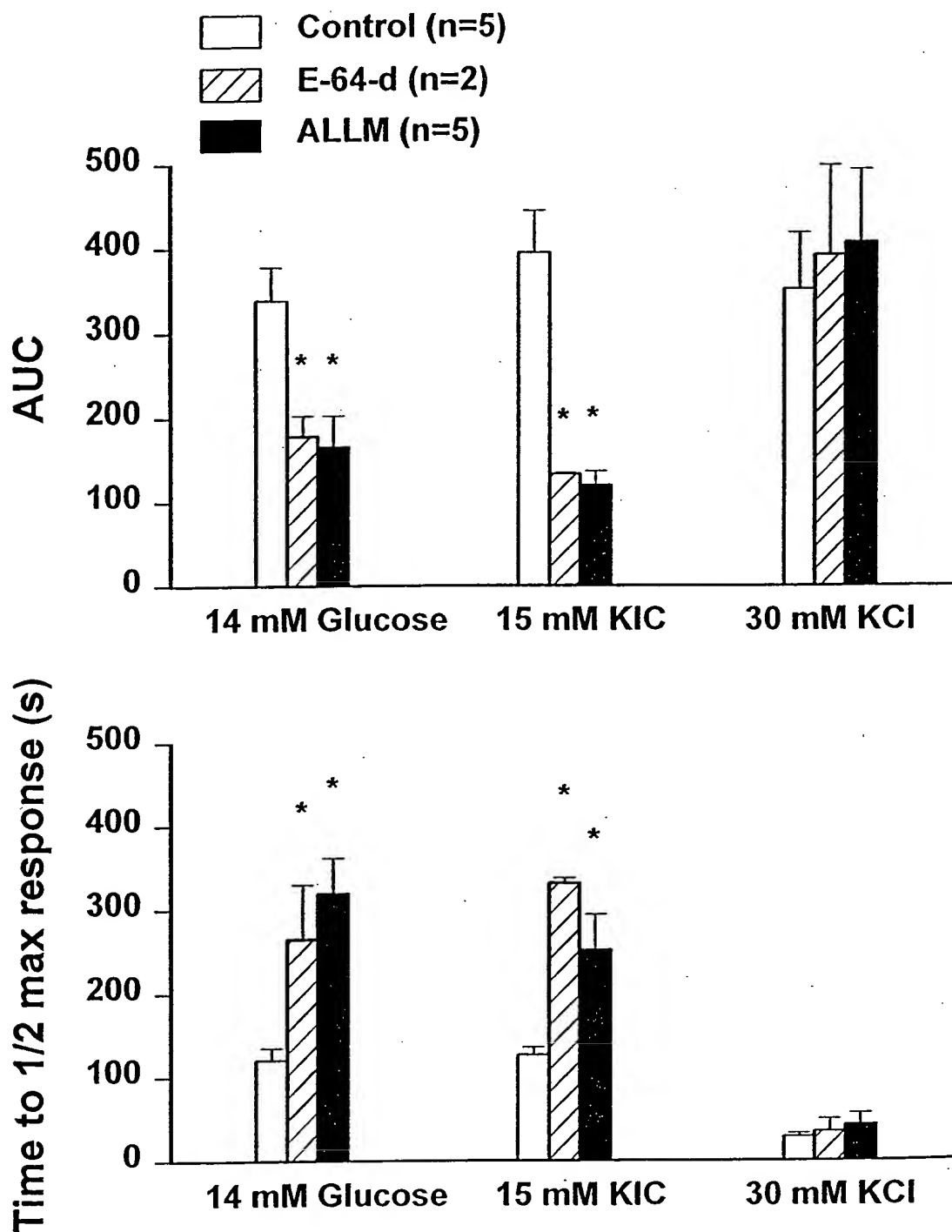


Fig. 20

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Fig 21. $[C^{2+}]_i$ responses to glucose, KIC and KCl



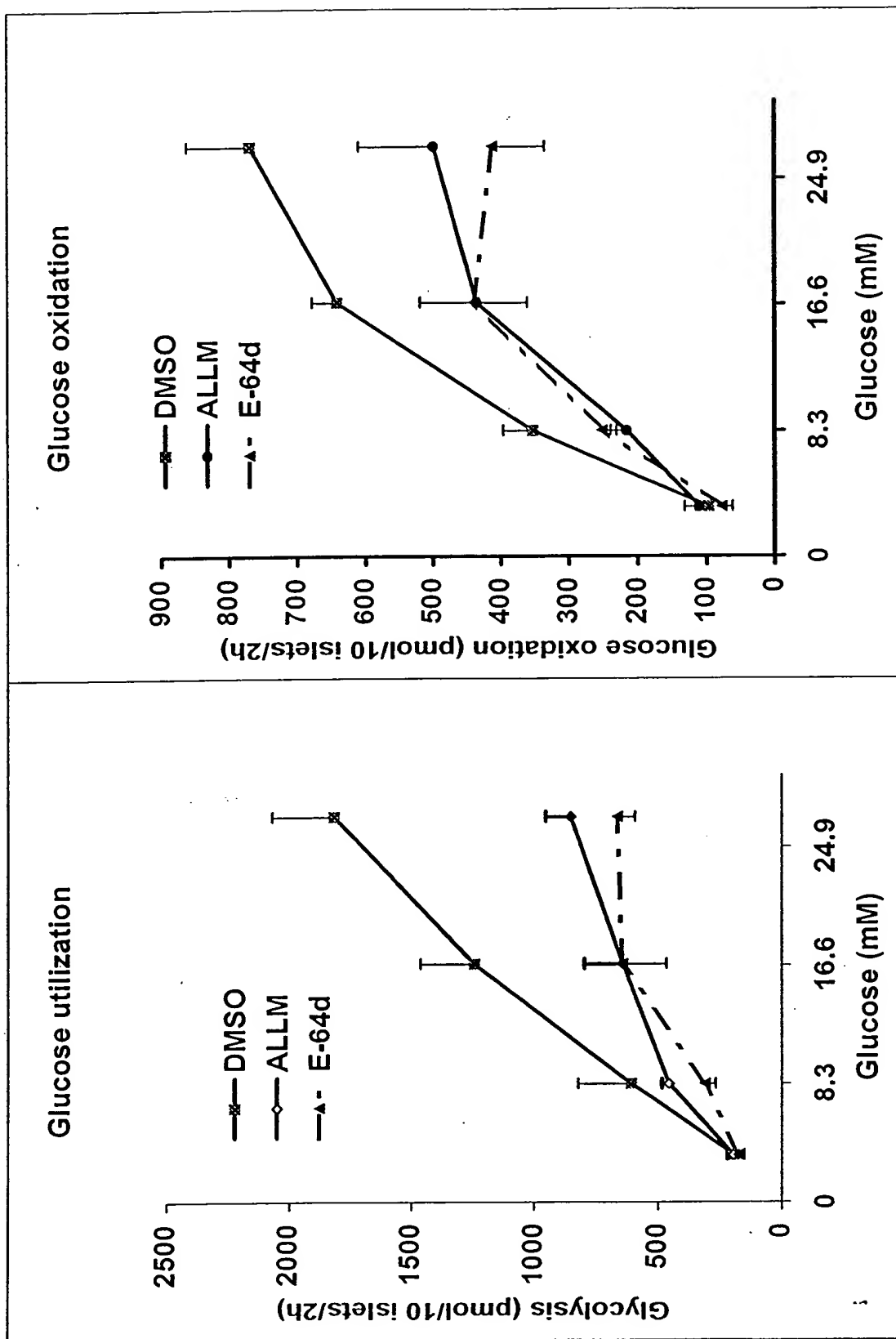
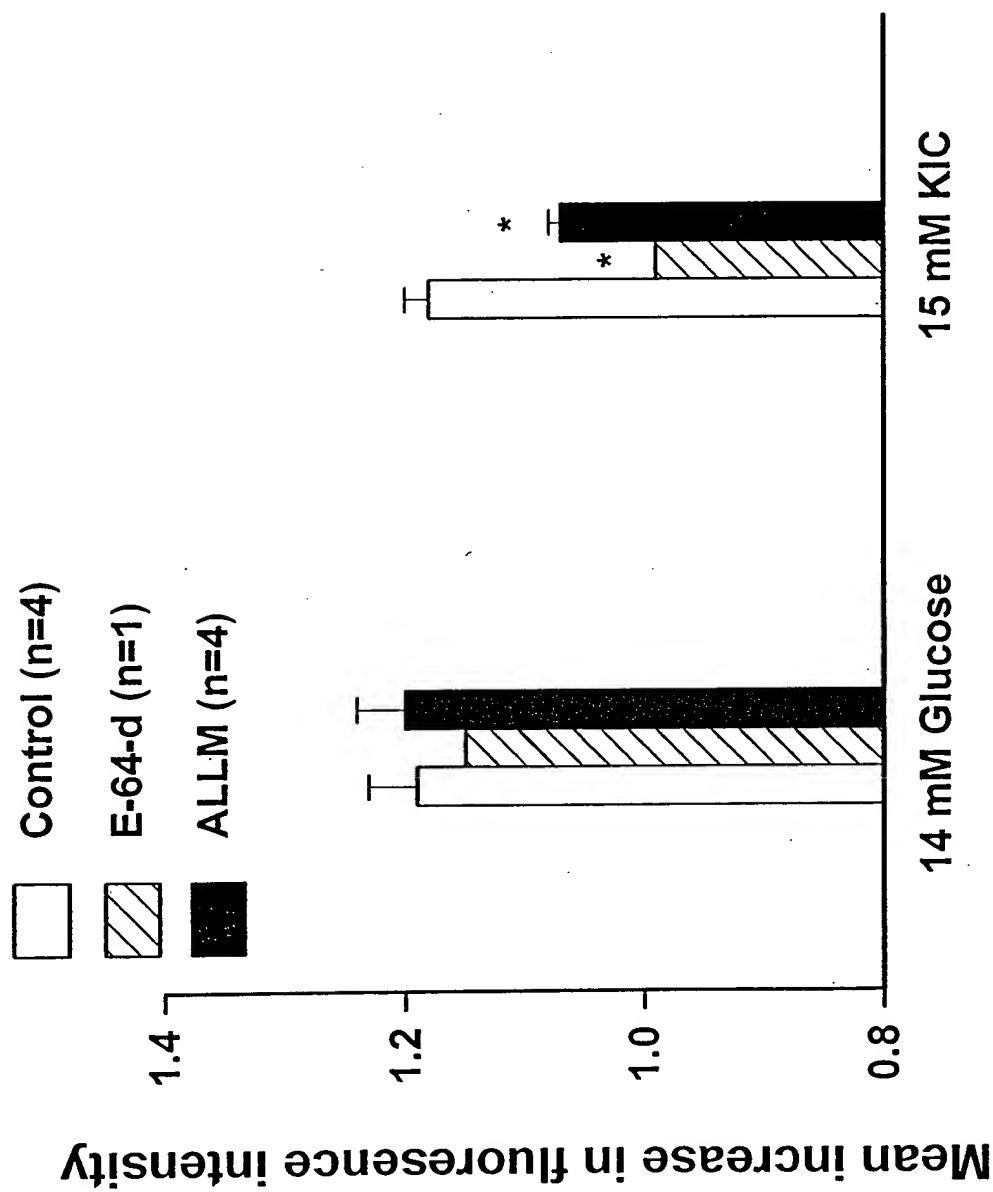


Fig. 22

Fig 23. NAD(P)H responses to glucose and KIC



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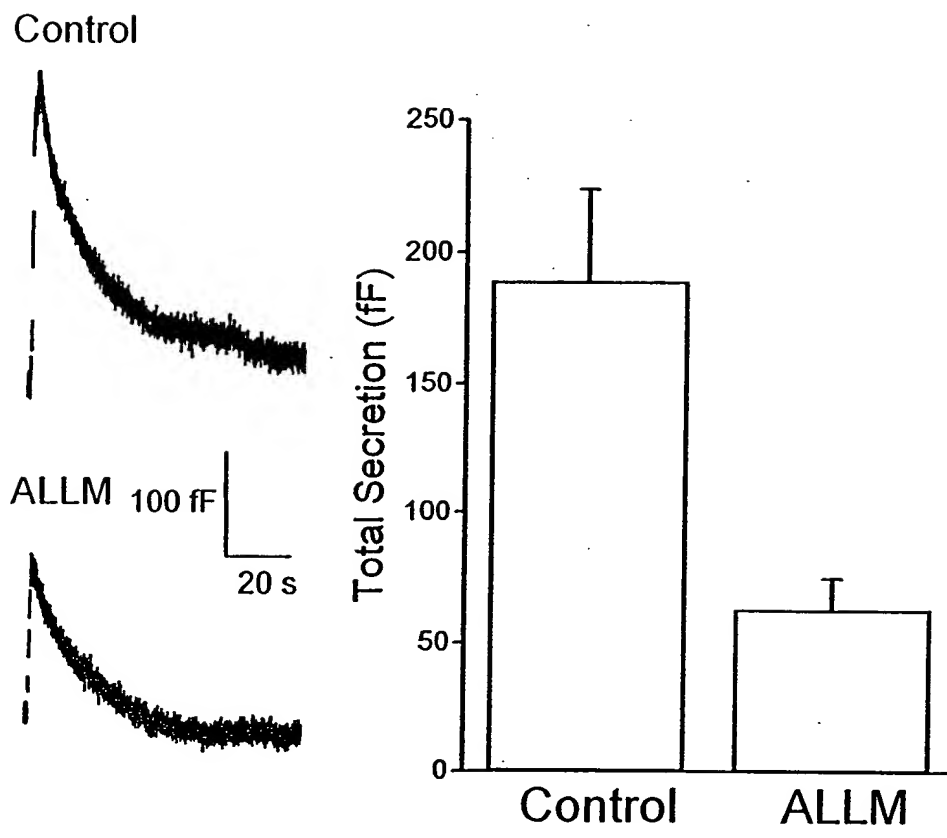
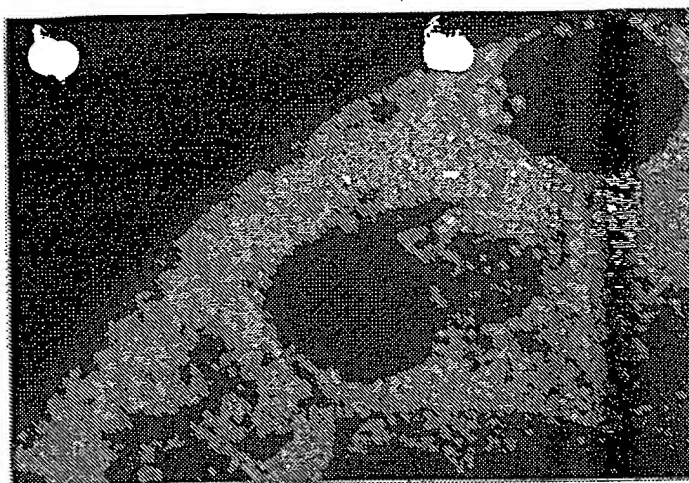


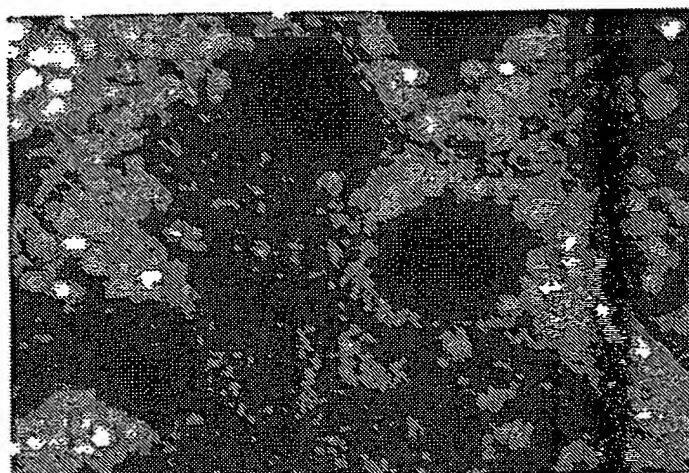
Fig. 24. Measurement of membrane capacitance in isolated β -cells

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DMSO



E64d



ALLM

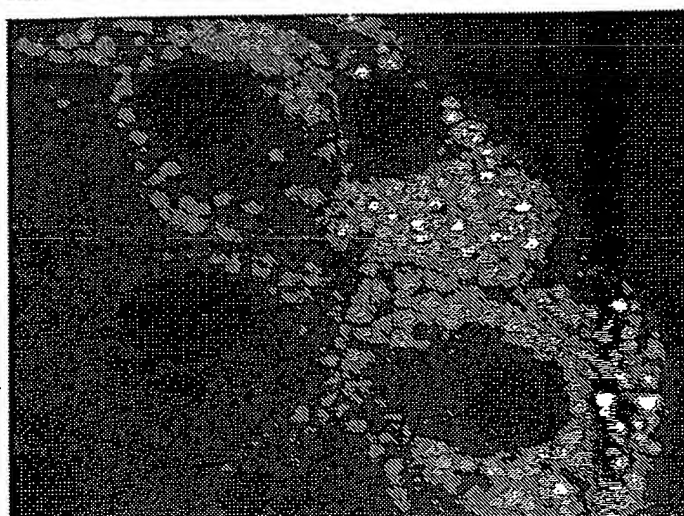


Fig. 25

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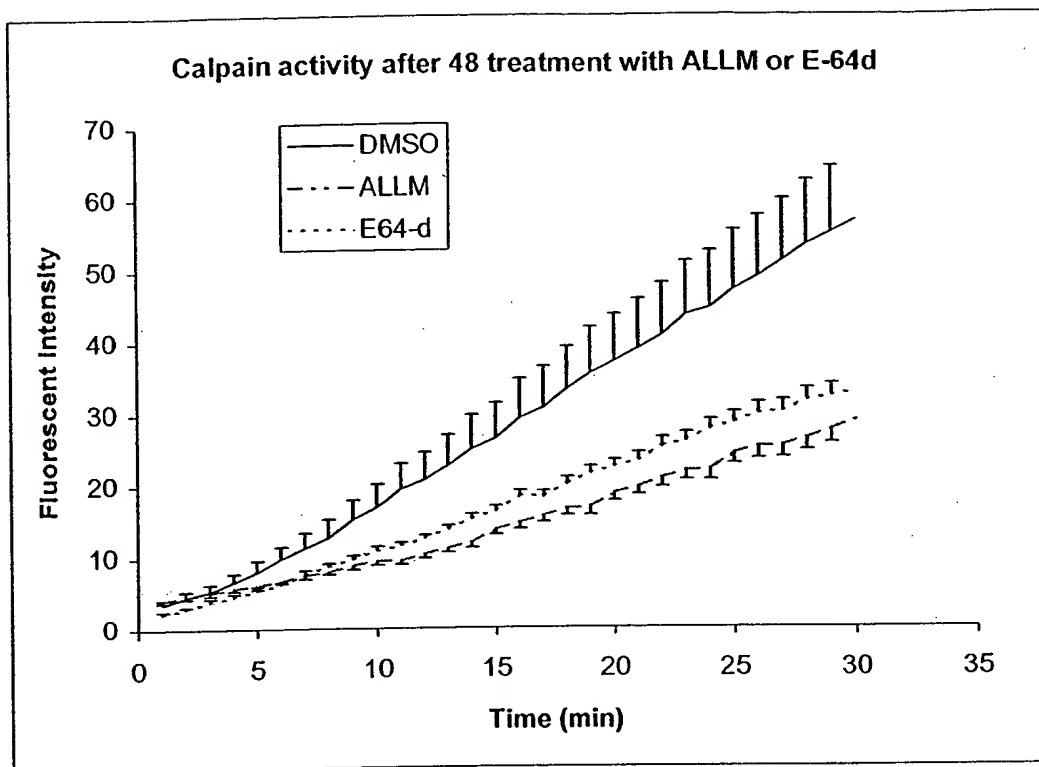


FIG. 26